Testing Penaeid Shrimp for Susceptibility to an Insect Nuclear Polyhedrosis Virus[1]

D. V. LIGHTNER,[2] R. R. PROCTOR,[2] A. K. SPARKS,[2]
J. R. ADAMS,[3] AND A. M. HEIMPEL[3]

ABSTRACT

Postlarval, early, and late juvenile stages of two species of penaeid shrimp, *Penaeus aztecus* Ives and *P. setiferus* (L.), were tested for susceptibility to a nuclear polyhedrosis virus from *Autographa californica* (Speyer). Shrimp were exposed to the virus by intramuscular inoculation of polyhedral protein-free virus and by feeding a diet containing virus polyhedra. Mortality attributable to viral infection did not occur during the 30-day test period, nor was there histological evidence of viral activity in shrimp hepatopancreas, gut, gill, striated muscle, ventral nerve, and segment nerve ganglia, or hypodermis.

Agricultural crops for several years have been sprayed or otherwise treated with chemical insecticides to reduce or eliminate insect pests. Chemical insecticides like DDT and other chlorinated hydrocarbons and organophosphates have been used routinely for this purpose. Because of the nonspecificity of these chemicals, the wide range of sensitive species, including desirable species, and the accumulation of some of these compounds in ecosystems, other more specific methods of controlling insect pests are preferred. Many methods of biological pest control such as introduction of predator species have been attempted. Knowledge in the area of biological control has continued to advance and control of some pest species by use of specific disease organisms may now be possible.

The USDA is involved in development of a biological control agent, a virus, which will infect and kill larvae of insect species representing 5 families of the Lepidoptera. Successful control of one of these species, the cabbage looper, *Trichoplusia ni* (Hübner), has been accomplished experimentally by spraying looper-infested cabbage crops with an insect nuclear polyhedrosis virus (NPV) (Jaques 1970, Splittstoessor and McEwen 1971).

Another experiment with the alfalfa looper nuclear polyhedrosis virus is planned for the Rio Grande Valley of Texas. Under certain conditions nuclear polyhedrosis viruses are stable, some retaining their infectivity for as long as 20 years (Bergold 1963). The virus may remain in the soil for long periods. Runoff could carry virus from treated agricultural areas into streams and eventually into estuaries of the Texas coast. In these areas postlarval shrimp develop through juvenile and subadult stages before returning to the Gulf of Mexico to complete their life cycle.

The objective of this study was to determine if penaeid shrimp, particularly the postlarval and early juvenile stages, can be infected by an insect NPV, and to determine the effect the virus has upon penaeid shrimp.

Materials and Methods

Virus

A sample of NPV of the alfalfa looper, *Autographa californica* (Speyer), was obtained from the Insect Pathology Laboratory USDA, Beltsville, Md. The virus was of known virulence for larvae of the alfalfa looper, with 1 insect lethal dosage being equal to 7 or 8 polyhedra/microliter of food. Two preparations of virus were made for infectivity studies.

The 1st preparation (stock solution A) consisted of a suspension of 5.2×10^9 polyhedra/ml of distilled water. The 2nd preparation (stock solution B) was a 3-ml suspension of virus rods, without polyhedral protein, prepared from 10 ml of stock solution A. The extraction and removal of polyhedral protein were accomplished by dissolution of polyhedral protein with a solution of 20 ml of 0.05 M sodium carbonate and 0.05 M sodium chloride. After a 30-min dissolution period, the total volume was increased to 160 ml by addition of distilled water. The resulting 160-ml suspension was centrifuged at 5000 rpm (Sorvall SS-4, SS-34 rotor)[4] for 15 min. The supernatant was decanted into a 2nd tube and centrifuged a 2nd time at 5000 rpm for 15 min. Pellets from the 1st and 2nd tubes were discarded. The supernatant from the 2nd tube was decanted into CO_2-free distilled water, then the resulting suspension of virus was centrifuged at 10,000 rpm for 1 h. The resulting pellet of virus rods was resuspended in CO_2-free distilled water and centrifuged again for 1 h at 10,000 rpm. The pelleted virus rods were resuspended in 3 ml distilled water. The approximate concentration of this solution (stock solution B) was 2.342×10^{12} virus rods/ml. This stock solution was diluted 1:100 in sterile saline prior to being tested on shrimp.

Experimental Animals

White shrimp, *Penaeus setiferus* (L.), juveniles

[1] Contribution no. 353, National Marine Fisheries Service Gulf Coastal Fisheries Center, Galveston Laboratory, Galveston, Tex. 77550. Received for publication Jan. 26, 1973.
[2] National Marine Fisheries Service, Gulf Coastal Fisheries Center, Galveston Laboratory, Galveston, Tex. 77550.
[3] USDA, Insect Pathology Laboratory, Beltsville, Md. 20705.

[4] Use of trade names does not imply endorsement of commercial products.

were obtained by trawl from West Bay near Galveston, Texas. Postlarval brown shrimp, *P. aztecus* Ives, were from artificially propagated stock at the Galveston Laboratory. Wild postlarval white shrimp and brown shrimp juveniles were not available in sufficient numbers to be tested at the time of these experiments (November–December 1971). The small juvenile white shrimp averaged 35.8 mm total length (measured from tip of rostrum to tip of telson) and 0.32 g weight, the larger juvenile white shrimp averaged 75.1 mm total length and 2.76 g weight, and the postlarval brown shrimp averaged 7 mm total length and 1.1 mg weight.

Postlarval brown shrimp were held in 4-liter glass jars containing 3 liters of Instant Ocean[5] artificial seawater at a rate of 10 shrimp/jar. The small juvenile white shrimp were held individually in 4-liter glass jars containing 3 liters of Instant Ocean, while the larger juvenile shrimp were held in 330-liter glass aquaria containing approximately 200 liters of Instant Ocean. Water in the glass jars was changed every 5 days during the 30-day experimental period but was not changed in the larger glass aquaria. Temperature and salinity were held constant in the jars and in the aquaria during the experimental period.

Methods of Inoculation

Test shrimp were inoculated with the virus by 2 routes: by intramuscular inoculation and by feeding of virus material. In the 1st method of inoculation with the virus, 50 white shrimp juveniles were given a 0.02-ml intramuscular injection of a virus suspension (solution B) containing 2.34×10^{12} virus rods/ml or about 4.7×10^{11} virus rods/shrimp. The injection was administered into the muscle between the 1st and 2nd abdominal segments using a sterile 1-ml tuberculin syringe with a 27-gauge needle. Shrimp exposed by this method were held in glass jars with Instant Ocean at 25°C and 20‰ salinity for 30 days. Fifty control shrimp received 0.02 ml of sterile saline without virus, but otherwise they were treated the same as those receiving virus. Control and test shrimp were fed boiled shrimp pieces daily. Tissue samples from shrimp inoculated with virus and control shrimp were taken for histological examination 5, 10, 20, and 30 days postinoculation. Tissue samples for electron microscopy were taken 30 days postinoculation. The experiment was terminated after 30 days.

The 2nd method of exposing shrimp to the virus was by mixing of viral polyhedra (solution A) with an artificial diet and feeding this diet to 100 postlarval, 35 small juvenile, and 15 large juvenile shrimp for 10 consecutive days. The food mixture consisted of ground whole shrimp, supplemental vitamins, and 2% gelatin by weight. Three diets were prepared: a control diet containing no virus, a 2nd containing 800 viral polyhedra/μl of food, and a 3rd containing 8000 viral polyhedra/μl of food. Mixing of virus into these diets was accomplished with a blender. All 3 diets were stored in a freezer until used to minimize bacterial growth.

Shrimp were fed daily at approximately 10% of their body weight. Generally, the shrimp ate all the food presented each day. Postlarval brown shrimp were fed the diet containing 800 polyhedra/μl of food, while the small juvenile and large juvenile white shrimp received the diet containing 8000 polyhedra/μl of food. Therefore, each experimental postlarval, small juvenile, and large juvenile shrimp received a total of approximately 8.8×10^{2}, 2.5×10^{6}, and 2.2×10^{7} polyhedra, respectively, during the 10-day feeding period. Control shrimp received the same diet during the 10-day period, but without virus. After the 10-day virus-feeding period, remaining test and control shrimp were fed boiled shrimp pieces for the remainder of the 30-day experimental period. All animals were held in Instant Ocean at 20°C and 20‰ salinity. As with the injection experiment, tissue samples for histological examination were taken from test and control groups on Days 5, 10, 20, and 30 of the experiment. Samples for electron microscopy were taken at the termination of the experiment (Day 30).

Virus-contaminated water was disinfected with chlorine at 1:2000 for at least 48 h prior to being discarded. Contaminated dip nets and other equipment were disinfected in a 1:200 solution of iodine in water.

Microscopy

Tissue samples for light microscopic examination were preserved in 10% phosphate-buffered formalin, decalcified by the formic acid method of Evans and Krajian (Krajian 1940), and embedded in paraffin. Tissue sections 8–10 μ in thickness were prepared, mounted on glass slides, and stained with hematoxylin and eosin.

Tissues for electron microscopy were given primary fixation in 5% phosphate-buffered glutaraldehyde (Sabatini et al. 1963) for 12 h at 4°C. After fixation, tissues were rinsed and stored in 0.15 M phosphate buffer (Millonig 1962) at 4°C until being mailed to the Insect Pathology Laboratory, for electron miscroscopic examination. At the Insect Pathology Laboratory, tissues were rinsed in 0.2 M phosphate buffer, postfixed in 1% osmium tetroxide in 0.2 M phosphate buffer, dehydrated in an ethanol series, and embedded in Spurr low-viscosity embedding medium (Spurr 1969). Sections were prepared with an LKB Ultratome III, double stained with uranyl acetate and lead citrate, and examined in a Hitachi-HU-11 electron microscope.

Results

Bioassay of Infectivity

Shrimp mortality attributable to viral infection by the NPV of *A. californica* did not occur in experiments in which the virus was fed or in experiments in which the virus was injected. In both experiments mortality in experimental and control groups was

[5] Aquarium System, Inc., 33208 Lakeland Blvd., Eastlake, Ohio 44094.

Table 1. — Accumulated mortalities in experimental and control groups of brown and white shrimp.

Shrimp	No. tested	No. dead after (accumulated mortality): 1 day	5 days	10 days	15 days	20 days	30 days	%
Brown postlarvae:								
Fed virus	100	–	7	11	–	12	29	29.0
Control	100	–	15	29	–	35	46	46.0
White juveniles:								
Fed virus	35	0	1	1	1	1	1	2.9
Control	35	0	3	3	4	4	5	14.3
Virus injected	50	3	5	5	5	5	5	10.0
Saline control	50	3	7	8	8	8	8	16.0
White subadults:								
Fed virus	15	0	0	0	0	1	3	20.0
Control	15	0	0	0	1	1	2	13.3

comparable (Table 1), indicating that the mortalities which did occur were due to factors other than the virus.

Microscopy

Tissues from experimental groups were examined for nuclear polyhedral bodies and were compared with the same tissues taken from control groups. From the experiment in which the virus was injected, 11 experimental and 11 control animals were examined histologically. The tissues or organs which received the most careful examination for polyhedra or other signs of viral activity were the hepatopancreas, gut, thoracic and abdominal striated muscle, ventral nerve and segment ganglia, gills, and hypodermis. Other tissues and organs such as heart, eye stalk, and gonads were frequently examined coincidentally with examination of the organs and tissues of primary interest. Nuclear polyhedra were not observed in the experimental group, nor were there apparent differences between the histological appearance of the organs and tissues of experimental and control groups.

From the experiments in which the virus was fed to experimental animals, 15 postlarval brown shrimp, 11 small juvenile, and 4 large juvenile white shrimp from the experimental groups were examined histologically for signs of viral infection. Fifteen postlarval brown shrimp, 11 juvenile, and 4 large juvenile white shrimp from the control groups in the feed experiments were examined histologically and compared with experimental groups. No signs of viral activity, polyhedra, or apparent histological differences were observed between experimental and control groups.

More than 30 samples of tissue were examined by electron microscopy. Tissues from the hepatopancreas, striated muscle, gills, and midgut of 11 shrimp (2 large juvenile white shrimp, 6 small juvenile white shrimp, and 3 postlarval brown shrimp) exposed to virus by injection or by feeding were examined for virus activity or presence of polyhedra. Comparable tissues from normal control shrimp not exposed to virus also were examined. No signs of virus activity or polyhedra were found in tissues of either experimental or control shrimp.

Conclusions

The nuclear polyhedrosis virus from *A. californica* appeared to be nontoxic and nonpathogenic for postlarval brown shrimp, *P. aztecus,* and juvenile white shrimp, *P. setiferus.* There were no significant differences in mortality between experimental groups injected with virus or fed virus and control groups. Furthermore, virus and virus polyhedra could not be demonstrated by light or electron microscopy in tissues taken from shrimp exposed to virus by injection or feeding.

REFERENCES CITED

Bergold, G. 1963. The nature of nuclear-polyhedrosis viruses, p. 413–56. *In* E. A. Steinhaus [ed.] Insect Pathology, An Advanced Treatise, Vol. 1. Academic Press, New York and London.

Jaques, R. P. 1970. Application of viruses to soil and foliage for control of the cabbage looper and imported cabbage worm. J. Invert. Pathol. 15: 328–40.

Krajian, Aram A. 1940. Histological Technique. The C. V. Mosby Co., St. Louis. 272 p.

Millonig, G. 1962. Further observations on a phosphate buffer for osmium solutions in fixation, p. 8. *In* S. S. Brease, Jr. [ed.] Proc. Fifth International Congress for Electron Microscopy, Vol. 2. Acadmic Press, New York.

Sabatini, D. D., K. Bensch, and R. J. Barrnett. 1963. Cytochemistry and electron microscopy: The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell. Biol. 17: 19–58.

Splittstoessor, C. M., and F. L. McEwen. 1971. Field evaluations of nuclear polyhedrosis viruses from the cabbage looper, *Trichoplusia ni.* J. Invert. Pathol. 17: 194–8.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26: 31–43.

Reprinted from the
ENVIRONMENTAL ENTOMOLOGY